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RADIOPROTECTION BY BIOLOGICAL RESPONSE MODIFIERS ALONE AND IN COMBINATION WITH WR-2721

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I. INTRODUCTION

Currently, protection against radiation exposure depends primarily on physical means such as shielding and avoidance. However, for individuals who might be required to engage in activities such as decontamination of fallout areas, clean-up of radiation accidents, or polar spaceflights, protection of physical means may be inadequate and/or impractical. In these situations, the availability of agents capable of providing protection against radiation injury would be of benefit. In addition, agents capable of protecting normal tissues from radiation injury and/or capable of enhancing the recovery of normal tissues postirradiation would assist patients undergoing aggressive radiotherapy.

Physiologically, the effects of radiation can be categorized into three syndromes (Walker, 1988). The hemopoietic syndrome occurs following the lowest radiation doses and results from irreversible damage to bone marrow hemopoietic stem cells. Under normal circumstances, these stem cells continually proliferate and differentiate to replace mature hematologic and immunologic cells being constantly lost through attrition. Following the loss of hemopoietic stem cells, hematologic and immunologic depletion rapidly occurs, and within a few weeks death ensues as a result of infection, hemorrhage and anemia (Talmage, 1955; Patt and Moloney, 1963; Taliaferro *et al.*, 1964).

Some substances, when administered prior to irradiation, have been recognized as being radioprotective specifically in the radiation dose range causing the hemopoietic syndrome. The best known of these substances is endotoxin (Mefferd *et al.*, 1953; Ainsworth, 1988), but impressive radioprotection also has been observed with *Bacillus Calmette Guerin* (BCG) and *Corynebacterium parvum*. All of these agents have been demonstrated to be potent nonspecific hemopoietic and immunologic stimuli. Optimal radioprotective effects usually have been observed with these agents administered 20–24 hr prior to irradiation. Because of this, the radioprotective mechanisms of these agents are thought to be different from those of traditional 'free-radical scavenging' sulfhydryl radioprotectors, which are most effective when administered within minutes of exposure. It has been suggested that the former agents mediate their radioprotective effects by mechanisms such as increasing the size of the preirradiation stem cell pools, synchronizing hemopoietic stem cells into less radiosensitive phases of the cell cycle, and/or by accelerating hemopoietic and immune repopulation from surviving stem cells postirradiation.

Ironically, substances found to be radioprotective specifically in the hemopoietic syndrome radiation dose range were never designed as radioprotectors (Torrence, 1985). The primary scientific interest in such agents, commonly referred to as immunomodulators or biological response modifiers (BRMs), was because of their potential therapeutic application in stimulating the immune system to fight cancer. Unfortunately, these BRMs also produced a variety of undesirable side effects, which precluded their use in man (Mansell and Klementz, 1973; Scott, 1974; Ribi, 1984). Over the past several decades (for reasons related to cancer therapy rather

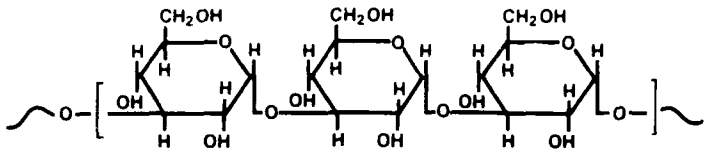

FIG. 1. Chemical structure of glucan, a polyglycan consisting of β-1,3-glucoside linkages.

than radioprotection), a great deal of time, energy and money has been dedicated to the discovery and/or development of new BRMs that are less toxic yet maintain potent immunomodulatory activity.

We have evaluated a variety of recently developed BRMs for the ability to enhance hemopoiesis and survival in irradiated animals (Patchen *et al.*, 1987a). This paper discusses the radioprotective effects of the BRM glucan, its modes of action, and the possibility of using glucan in combination with other agents to further protect and/or enhance recovery from radiation injury.

2. GLUCAN: BACKGROUND AND GENERAL IMMUNOLOGIC AND HEMOPOIETIC EFFECTS

Glucan (Fig. 1) is a beta-1,3-polyglucose isolated from the inner cell wall of the yeast *Saccharomyces cerevisiae* (Hassid *et al.*, 1941; DiLuzio *et al.*, 1979). Upon injection, glucan appears to be specifically taken up by macrophages (Gilbert *et al.*, 1977). Monocytes and macrophages recently have been demonstrated to possess glucan receptors (Czop and Austin, 1985; Abel *et al.*, 1987); hence, glucan's effects may be receptor-mediated. It has been recognized for many years that glucan is a potent macrophage activator (Wooles and DiLuzio, 1963, 1964; DiLuzio *et al.*, 1970). In addition, both primary and secondary immune responses and a variety of cell-mediated immune responses have been shown to be enhanced following glucan administration (Wooles and DiLuzio, 1963; DiLuzio, 1967; Cook *et al.*, 1978). These later effects are suspected of resulting via cytokine-mediated cascades initiated following macrophage activation. In addition to these immunological effects, glucan also has been shown to dramatically enhance hemopoiesis at the pluripotent stem cell and committed progenitor cell levels (Patchen and Lotzova, 1980); Patchen and MacVittie, 1983).

Original glucan preparations were particulate in nature and because of this, glucan's clinical potential was limited. In recent years, however, soluble glucan preparations have been developed and have been demonstrated to induce immunologic and hemopoietic effects similar to those observed with the original participate glucan preparations (Patchen and MacVittie, 1986a,b).

3. GLUCAN-MEDIATED RADIOPROTECTION

Based on the ability to enhance survival in otherwise lethally irradiated mice, both participate and soluble glucan have been shown to be radioprotective (Patchen, 1983; Patchen and MacVittie, 1986b; Patchen *et al.*, 1986, 1987b). This effect is dependent on the route of glucan injection, the glucan dose and the time elapsed between glucan injection and irradiation. Optimal radioprotection is generally observed with glucan injected intravenously approximately 20 prior to irradiation (Fig. 2).

In sublethally irradiated mice, it has been demonstrated that glucan accelerates the repopulation of both pluripotent hemopoietic stem cells (CFU-s) and committed granulocyte-macrophage (GM-CFC), pure macrophage (M-CFC), and erythroid (CFU-e, BFU-e) hemopoietic progenitor cells (Patchen and MacVittie, 1982; Pospisil *et al.*, 1982; Patchen, 1983; Patchen *et al.*, 1984a,b). This has been shown to occur with glucan administered either 1 day before, 1 hr before, or even 1 hr after irradiation. However -1 day glucan injection was generally more effective than -1 hr injection, and -1 hr injection generally more effective than $+1$ hr injection.

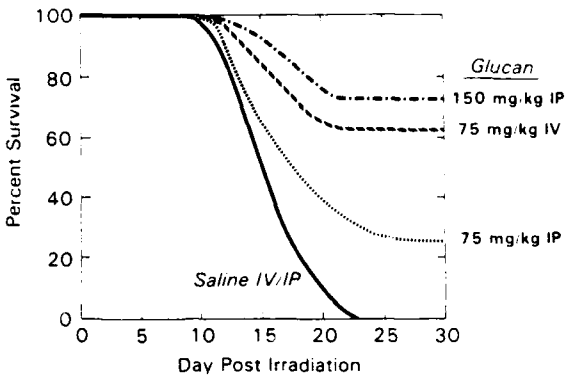

FIG. 2. Survival-enhancing effects of glucan. C3H/HeN mice were injected with saline, glucose (75 mg/kg), or glucan (75 or 150 mg/kg) ~20 hr prior to receiving 9.0 Gy ^{60}Co radiation. Data represent cumulative survival data obtained from 45–61 mice in each treatment group.

TABLE 1. *Effect of Glucan on Survival and on Pluripotent Hemopoietic Stem Cell Recovery in Irradiated Mice**

Day post irradiation	Percent survival		CFU-s/femur† (percent control)		CFU-s/spleen‡ (percent control)	
	Saline	Glucan	Saline	Glucan	Saline	Glucan
11	96	99	0	0	0	0
13	80	95§	0	0	0	0.51 ± 0.08§
15	49	86§	0	0.15 ± 0.09§	0.02 ± 0.02	0.68 ± 0.12§
18	18	75§	—¶	0.96 ± 0.16	—¶	12.94 ± 0.96
21	0	72§	—¶	2.56 ± 0.31	—¶	20.58 ± 2.06

*C3H HeN mice were injected i.v. with either saline or particulate glucan (75 mg/kg) ~20 hr prior to receiving 9.0 Gy ^{60}Co radiation.

†The number of CFU-s per femur in normal control mice was 1655 ± 55.

‡The number of CFU-s per spleen in normal control mice was 3315 ± 97.

§Compared to saline control values, $p < 0.05$.

¶Too few surviving mice to adequately evaluate at these time points.

In otherwise lethally irradiated mice, glucan also accelerates CFU-s and GM-CFC recovery (Patchen *et al.*, 1987a,b; Table 1). However, at day 21 postirradiation (when all radiation control mice have died, and after which no glucan-treated mice die), the bone marrow and splenic CFU-s contents of surviving glucan-treated mice contain only 2.56% and 20.58%, respectively, of the CFU-s contents observed in normal control mice. Furthermore, no hemopoietic recover is detected until day 13 postirradiation. This, coupled with the fact that as early as 9 days postirradiation opportunistic bacterial infections occur less frequently in glucan-treated mice than in radiation control mice (Patchen *et al.*, 1986, 1987a,b; Table 2), suggests that glucan-mediated radioprotection involves mechanisms other than those solely related to hemopoietic regeneration.

Macrophages, which have been shown to be activated following glucan administration, have also been shown to be some of the most radioresistant of all hemopoietic and immunologic cells (Gallin and Green, 1987). In addition, these cells play a major role in host defense against microbial invasion and synthesize and release cytokines capable of stimulating hemopoietic proliferation and differentiation (Griffin, 1982; Reichard and Filkens, 1984). In light of this, we suspected that glucan's initial survival-enhancing effects may be macrophage-mediated. Experiments in which 5'-nucleotidase activity (an ectoenzyme whose activity decreases with macrophage activation) was used as an indicator of macrophage activation revealed that although macrophages from both saline-treated and glucan-treated mice become activated within 1 hr after irradiation, macrophages from saline-treated mice soon revert to an unactivated state, while those in glucan-treated mice remain activated for several weeks (Patchen *et al.*, 1987b; Fig. 3).

TABLE 2. *Effect of Glucan on Survival and on Splenic Bacterial Translocation in Irradiated Mice**

Day post-irradiation	Percent survival		Percent of surviving mice exhibiting splenic bacterial translocation†	
	Saline	Glucan	Saline	Glucan
7	100	100	12.2 ± 0.9	10.0 ± 0.7
9	100	100	32.2 ± 1.4	15.8 ± 0.9 ‡
11	96	99	41.0 ± 1.8	22.4 ± 1.1 ‡
13	80	95 ‡	57.2 ± 3.1	10.1 ± 0.8 ‡
15	49	86 ‡	80.1 ± 4.9	6.9 ± 0.4 ‡
18	18	75 ‡	—§	5.7 ± 0.4
21	0	72 ‡	—§	4.1 ± 0.3

*C3H/HeN mice were injected i.v. with either saline or particulate glucan (75 mg/kg) ~20 hr prior to receiving 9.0 Gy ^{60}Co radiation.

†Organisms detected were *Proteus mirabilis*, *Escherichia coli* and *Staphylococcus aureus*.

‡Compared to saline control values, $p < 0.05$.

§Too few surviving mice to adequately evaluate at these time points.

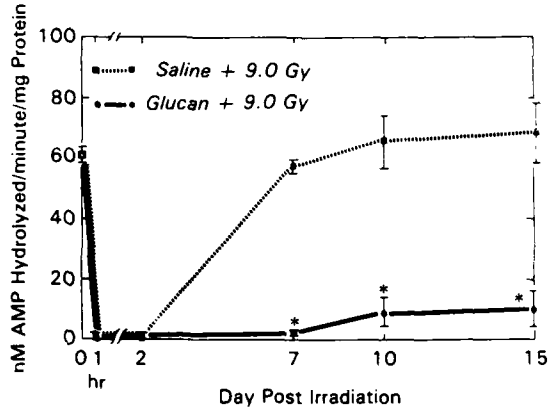

FIG. 3. 5'-Nucleotidase activity in peritoneal macrophages obtained from C3H/HeN mice injected i.p. with either saline or particulate glucan (75 mg/kg) ~20 hr prior to 9.0 Gy ^{60}Co radiation. Each data point represents the mean ± standard error of values obtained from 3–4 experiments, each performed with cells pooled from 8–10 mice. Statistical differences were determined by Student's *t*-test; * represents $p < 0.05$ with respect to control mice.

The role of macrophages in mediating BRM-induced radioprotection is also suggested by hemopoietic and survival data obtained with a variety of other BRMs. In a comparison of 17 BRMs, agents whose primary target cells included macrophages generally were more capable of enhancing hemopoiesis in irradiated mice than BRMs whose primary target cells did not include macrophages (Patchen *et al.*, 1987a). Furthermore, only BRMs whose primary target cells included macrophages were capable of enhancing survival in otherwise lethally irradiated mice (Patchen *et al.*, 1987a; Chirigos and Patchen, 1988).

4. RADIOPROTECTIVE COMBINATIONS

We have recently suggested that safer and more effective radioprotection may be achieved by combining nontoxic doses of several radioprotectors that function via different mechanisms. Examples of several such combinations are described below.

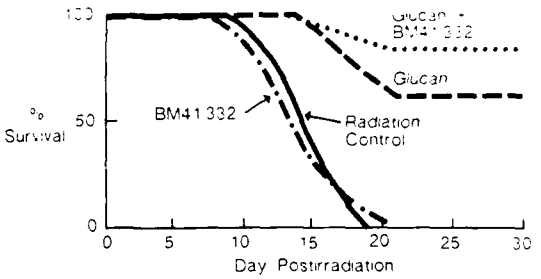

FIG. 4. Effects of combined BM41.332 and glucan administration on survival of irradiated C3H/HeN mice. Approximately 20 hr prior to 9.0 Gy ^{60}Co radiation, mice were injected i.v. with particulate glucan (75 mg/kg), BM41.332 (10 mg/kg), or both substances. Data represent cumulative survival data obtained from 47–77 mice in each treatment group.

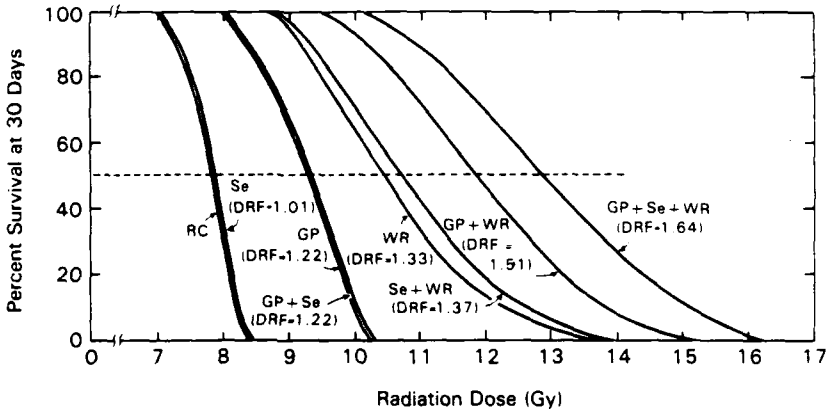

FIG. 5. Effects of combined glucan, selenium and WR-2721 administration on survival in irradiated C3H/HeN mice. Particulate glucan (GP, 75 mg/kg, i.v.), and selenium (Se, 0.8 mg/kg as sodium selenite, i.p.) were administered ~20 hr prior to irradiation. WR-2721 (WR, 200 mg/kg, i.p.) was administered ~30 min prior to irradiation. RC = saline-injected, irradiated mice. Data represent cumulative survival data obtained from 51–673 mice in each treatment group.

4.1. BRM–BRM Combinations

Radioprotective BRM combinations have been created using BRMs that are targeted at different cell populations. One such example is the use of glucan in combination with 2-cyano-1-[(2-methoxy-6-methyl-pyridin-3yl)-methyl]-aziridine (BM41.332). BM41.332 is not at all radioprotective when administered alone. However, when combined with a radioprotective dose of glucan, a synergistic survival enhancement approximately 20% greater than that obtained with glucan alone is observed (Fig. 4). Synergistic or additive radioprotective effects also have been obtained recently using combinations of immunomodulating cytokines (Neta *et al.*, 1987). However, in spite of the fact that BRM–BRM combinations have enhanced survival beyond that observed with single-agent BRM treatments, the dose reduction factors (DRFs) obtained with such combinations still have been limited to ~1.2–1.3. This suggests that BRMs protect primarily cells comprising the hemopoietic and immune systems.

4.2. BRM–Aminothiol Combinations

In an attempt to obtain DRFs greater than 1.2–1.3, BRMs have been combined with other more generalized radioprotectors. The aminothiol WR-2721 (ethiofos) has been demonstrated to be one of the most potent radioprotectors available (Brown *et al.*, 1988). When administered

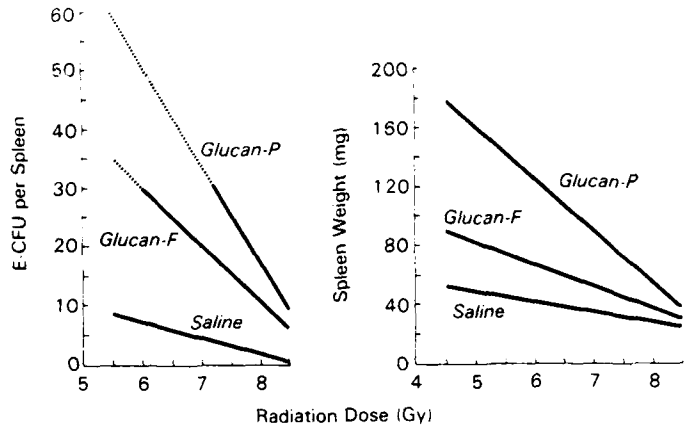

FIG. 6. Effect of postirradiation glucan administration on endogenous spleen colony formation (E-CFU) and spleen weight. C3H/HeN mice were exposed to the indicated doses of ^{60}Co radiation and 1 hr later intravenously injected with saline, particulate glucan (Glucan-P, 75 mg/kg), or soluble glucan (Glucan-F, 250 mg/kg). Twelve days later, the spleens were removed, fixed in Bouin's solution, weighed, and the number of E-CFU counted. Data were plotted and computer-generated extrapolation lines drawn through the data points. Dotted portions of lines indicate radiation doses at which confluent E-CFU formation was observed.

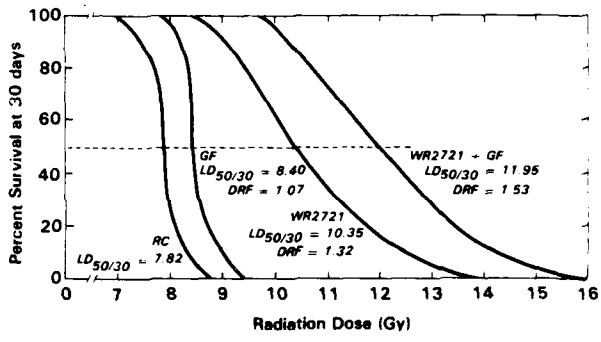

FIG. 7. Survival-enhancing effects of postirradiation glucan administration combined with preirradiation WR-2721 administration. C3H/HeN mice were injected i.p. with WR-2721 (200 mg/kg) ~30 min prior to irradiation and injected i.v. with soluble glucan (GF, 250 mg/kg) ~1 hr after irradiation. Data represent cumulative survival data obtained from 75–623 mice in each treatment group.

shortly before irradiation, WR-2721 protects a variety of tissues from radiation injury; however, the best effects have been observed in hemopoietic tissues. Unfortunately, the radioprotective effects of WR-2721 are directly dose-dependent, and WR-2721 doses that produce the best radioprotection also induce nausea, vomiting and other potentially more dangerous side effects (Cairnie, 1983); Kligerman *et al.*, 1984). We have attempted to reduce side effects and yet maintain good radioprotection by administering nontoxic doses of WR-2721 in combination with BRMs. Figure 5 illustrates the results of survival studies in which glucan and a low dose of WR-2721 were used. The DRF of 1.51 obtained with the combination of these two agents is additive between that obtained with glucan (DRF 1.22) and that obtained with low-dose WR-2721 (DRF 1.33). If selenium, which has been shown to reduce the endogenous toxicity of WR-2721 (Weiss *et al.*, 1987), is also administered ~20 hr prior to irradiation, a DRF of 1.64 is observed (Fig. 5). These studies demonstrate the feasibility of using nontoxic doses of several agents to additively and/or synergistically produce DRFs greater than 1.2.–1.3.

Another approach to the use of glucan in combination with WR-2721 has been its administration postirradiation. This approach is based on our observation that, even when administered postirradiation, glucan can significantly increase hemopoietic stem cell numbers (Fig. 6). Thus,

TABLE 3. *Effect of WR-2721 and Glucan on Endogenous Hemopoietic Spleen Colony Formation (E-CFU) and on Spleen Weight**

	10 Gy		11 Gy		12 Gy	
	E-CFU†	SPL WT‡	E-CFU†	SPL WT‡	E-CFU†	SPL WT‡
Radiation control	0.04	14.5	0.00	13.0	0.00	12.9
WR-2721	7.05	25.8	2.00	19.5	0.74	16.5
Glucan	0.20	16.5	0.01	16.3	0.00	16.0
WR-2721 + Glucan	24.47	59.5	13.42	39.5	4.65	23.2

*C3H/HeN mice were injected i.p. with saline or WR-2721 (200 mg/kg) ~30 min prior to irradiation and injected i.v. with saline or soluble glucan (250 mg/kg) ~1 hr after irradiation.
†E-CFU were counted 12 days after irradiation.
‡Weight in mg.

if low-dose WR-2721 treatment prevents the destruction of even a very few hemopoietic stem cells, postirradiation glucan therapy should be capable of stimulating the proliferation of these cells and result in accelerated hemopoietic recovery and increased survival. Results supporting this hypothesis and demonstrating synergistic hemopoiesis- and survival enhancing effects of preirradiation low-dose WR-2721 treatment combined with postirradiation glucan treatment are illustrated in Fig. 7 and Table 3. Interestingly, multiple postirradiation glucan injections did not enhance survival beyond that observed with a single glucan injection given 1 hr after exposure (Patchen, unpublished results).

5. GENERAL COMMENTS AND CONCLUSIONS

It is clear that even when used alone glucan can function protectively and/or therapeutically in radiation-injured host. When used in this manner, 'radioprotective' potential appears to be limited to DRFs of 1.2–1.3. However, when used in combination with even low doses of traditional aminothiol radioprotectors such as WR-2721, glucan can additively or synergistically increase DRFs to 1.5–1.6. Such results suggest that not only better radioprotection but also reduced toxicity may be obtained by using low-to-modest doses of several radioprotective agents that act via different mechanisms. Furthermore, based on measurements of motor performance in mice, glucan even appears to reduce the behavioral toxicity of WR-2721 (Landauer and Patchen, manuscript in preparation).

Glucan is only one of several macrophage-activating BRMs that have been demonstrated to be both hemopoietic stimulants and radioprotectors (Patchen *et al.*, 1987a). Numerous other BRMs remain to be evaluated for these effects, and they may prove to be as good as or better than glucan as radioprotectors. As more data accumulate, it appears that these agents (alone or in combination with other agents) are establishing their usefulness in the treatment and/or prevention of acute radiation injury.

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